

# Ion channels in plants

## From bioelectricity, via signaling, to behavioral actions

František Baluška<sup>1\*</sup> and Stefano Mancuso<sup>2</sup>

<sup>1</sup>University of Bonn; IZMB; Bonn, Germany; <sup>2</sup>University of Florence; LINV; Sesto Fiorentino, Italy

In his recent *opus magnum* review paper published in the October issue of *Physiology Reviews*, Rainer Hedrich summarized the field of plant ion channels.<sup>1</sup> He started from the earliest electric recordings initiated by Charles Darwin of carnivorous *Dionaea muscipula*,<sup>1,2</sup> known as Venus flytrap, and covered the topic extensively up to the most recent discoveries on Shaker-type potassium channels, anion channels of SLAC/SLAH families, and ligand-activated channels of glutamate receptor-like type (GLR) and cyclic nucleotide-gated channels (CNGC).<sup>1</sup>

It is interesting to recall that the earliest electric recordings on animal (squid neurons) cells were studied side-by-side with plant (algae *Nitella* and *Chara*) cells. Pioneers of bioelectricity discovered that the plasma membrane of all eukaryotic cells is excitable and electrically active, despite some differences in ions and ion channels involved.<sup>1</sup> Some cells, especially neurons, are electrically more active and communicate via action potentials. Surprisingly, although very similar action potentials were recorded in plants already in 1873, the role of plant action potentials is still not settled in today. Nevertheless, it is obvious that not only the so-called sensitive plants, but in fact all plants generate action potentials (APs) and that these APs serve for communication and integration of plant bodies,<sup>3-5</sup> which can attain extraordinary sizes in some trees. Asymmetric distributions of ions at cell periphery generates resting electric potential at the plasma membrane.<sup>6</sup> This is about -100 mV in typical animal cells<sup>6-8</sup>

but higher in most plant cells, sometimes exceeding even -200 mV.<sup>9</sup> Interestingly, this resting electric potential at the plasma membrane is changing also along the root apex.<sup>10</sup> The next more complex feature of plant cells, in comparison to animal cells, is the presence of the vacuolar membrane which is also equipped with ion channels and generates its own electrochemical gradient.<sup>1,11</sup> This results in trans-cytoplasmic potential of about -100 mV.<sup>1</sup>

With respect to Venus flytrap, we know now that a single mechanical stimulus of sensory hairs induces an action potential (AP),<sup>12</sup> but the trap requires two repetitive stimuli (APs) to close and three repetitive APs are required to activate digestive glands.<sup>13</sup> Moreover, Venus flytrap uses electrical memory to control the behavior of its trap.<sup>14,15</sup> *Dionaea muscipula* traps and *Mimosa pudica* leaves get immobilized when exposed to anesthetics.<sup>16,17</sup> Importantly, they can be easily recovered to behavioral activity by removing anesthetics from their environment. All this suggest fascinating similarities between plants and animals with respect of APs driving motoric behavior. Intriguingly, numerous examples of sophisticated plant behavior implicate existence of plant-specific consciousness and intelligence too.<sup>19</sup>

Surprisingly, recent genomic studies have revealed unexpected ligand-activated channels of GLR and CNGCs families; and, moreover, these animal-like channels outnumbered the well-studied potassium channels. For example, there are 15 potassium channels in *Arabidopsis*, but as many as 20 GLRs and 20 CNGCs.<sup>18</sup> Even greater differences have been scored in larger and more complex plants like

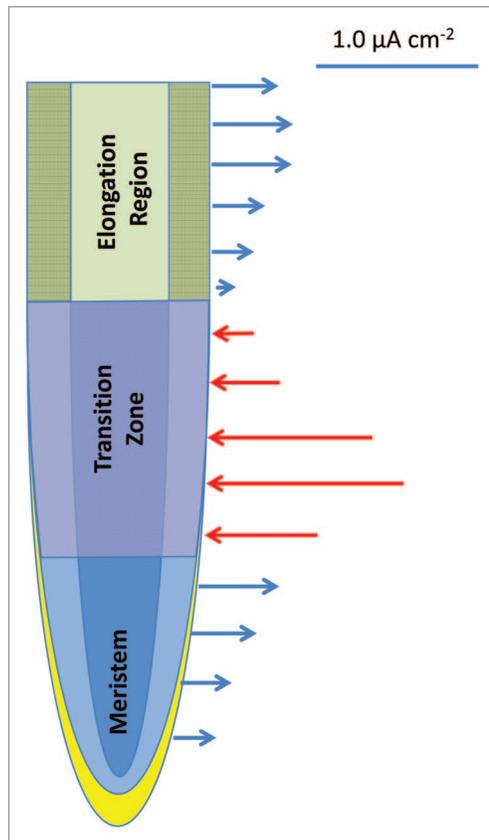
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\*Correspondence to: František Baluška;  
Email: baluska@uni-bonn.de



**Figure 1.** Schematic visualization of the electric field around the growing maize root apex using data published by Collings et al.<sup>28</sup> The outward current is shown by blue arrows, the inward current is depicted by red arrows. The two reversals of the electric current polarity at the both borders of the transition zone are typical for root apices of all plants tested so far. Identity of ion channels underlying this phenomenon is enigmatic so far, but they do not seem to be related to fluxes of H<sup>+</sup> and K<sup>+</sup>. The GLRs and CNGCs are strong candidates in this respect.

poplar tree: 15 potassium channels vs. 61 GLRs.<sup>20</sup> Perhaps the greatest mystery is associated with the plant GLRs. Although they were discovered more than ten years ago, we still know very little about their localization and function. If these are used in analogy to the animal/neuronal GLRs then one could expect that plant cells assemble synaptic domains specialized for cell-cell communication, analogously to neurons in brains. Accordingly, root apex cells in the transition zone not only shows F-actin-based adhesion domains specialized for endocytosis, endocytic vesicle recycling, and cell-cell communication; but they also show high rates of APs and synchronous electrical firing.<sup>21-23</sup> In neurons organized into brains, cell-cell communication is based on endocytic recycling and regulated exocytosis of synaptic vesicles, when synaptotagmin acts as calcium sensor.<sup>24</sup> Intriguingly in

this respect, plant-specific synaptotagmin AtSYT1 localizes to the plant-specific synaptic domains of Arabidopsis root apices too,<sup>25</sup> and controls besides exocytosis also endocytosis.<sup>26</sup> Our preliminary data suggest that Arabidopsis GLRs control endocytic vesicle recycling in the transition zone cells of root apices of young Arabidopsis seedlings (Matthias Weiland, Boris Voigt and František Baluška, unpublished data).

Activities of ion channels generate electric fields around the plasma membrane, whereas a sum of all ion channels activities in a given zone of the root apex is responsible for patterns of electric fields around the whole root apex,<sup>27,28</sup> like in animals and humans. For example, in human brains, summations of synchronous ion channel activities of all neurons generate characteristic electric fields which can be measured using electroencephalography (EEG).<sup>29,30</sup> Although these extracellular electric fields

around plant roots are generated by H<sup>+</sup> and K<sup>+</sup> fluxes, activities of ligand-activated ion channels are also involved in the generation of brain EEG.<sup>29</sup> In the growing root apices of all plants tested, there are three stable distinct zones of electric current flow (Fig. 1): outward currents are recorded around the root cap, meristem and the elongation region, whereas prominent inward currents are scored at the transition zone.<sup>27,28</sup> Thus, there are two sudden reversals of the electric current polarity around growing root apex: one is the outward - inward switch when cells cease mitotic divisions and enter the transition zone; another one is the inward - outward switch when cells leave the transition zone and enter the region of rapid cell elongation.<sup>28</sup> Currently, there is no explanation for these sudden reversals of the electric current polarity in the transition zone<sup>31-35</sup> of the root apex. Transport activities are not only synchronized in the transition zone, but show also oscillations.<sup>37-40</sup> In addition, root apex electric fields<sup>41,42</sup> and gene expression activities<sup>43,44</sup> are also known to oscillate in this root apex zone. In future, it will be important to test whether activities of the GLRs and CNGCs are behind these intriguing aspects of the root apex transition zone. The fact that all GLRs, and most of the CNGCs, are expressed in roots of Arabidopsis<sup>18,36</sup> is particularly relevant in this respect.

Recently, the first breakthrough studies have started to illuminate the elusive functions of GLRs in plants. First of all, Michard et al.<sup>45</sup> reported that the plant GLRs in pollen tubes of Arabidopsis and tobacco act similarly as the neuronal glutamate receptors. They are activated by their ligands to transport calcium into cells in a signaling-like mode, allowing a signal-mediated calcium influx, which then controls pollen tube tip-growth and navigation.<sup>45</sup> Interestingly, GLRs in the tip-growing pollen tube are activated via D-serine, which is released from pistil tissues. D-serine is produced from L-serine via Arabidopsis serine-racemase SR1.<sup>45</sup> This milestone paper suggests that pollination in plants is a neuronal-like process in which pollen tubes are navigated through pistil tissues via neuronal-like cell-cell communication. This view is

supported also by previous papers showing that navigation of pollen tubes is also controlled by NO and GABA.<sup>46-49</sup> Hence, it is particularly relevant that GABA-binding proteins have been detected at the pollen plasma membrane which are involved in calcium transport and oscillations.<sup>50</sup>

Plant GLRs are now recognized to have also important role in plant–pathogen interactions. It is well known that diverse pathogens induce calcium fluxes and generate calcium spikes in plant cells exposed to pathogens or relevant microbe-associated molecular pattern (MAMPs) such as flagellar proteins, chitin fragments, elongation factors, or secreted peptide elicitors. These MAMPs are then recognized by cell surface receptors, which signal downstream via calcium influx to alert the attacked cells. For example, flagellin is recognized by the receptor kinase FLS2, which interact with BAK1 (BR1-associated receptor kinase 1)<sup>51</sup> to activate calcium influx, rapid plasma membrane depolarization and H<sub>2</sub>O<sub>2</sub> burst.<sup>1</sup> All this happens within the first two minutes of flagellin exposure.<sup>1</sup> Intriguingly, plant GLRs are participating in this MAMP-triggered calcium influx,<sup>52,53</sup> and analogous to mammalian NMDA-NR1 receptor, calmodulin is also involved.<sup>53</sup> The next breakthrough is the discovery that plant GLRs are activated by elicitor cryptogein, being partly responsible for cryptogein-induced NO production.<sup>54</sup>

Intriguingly, cryptogein-induced vesicular secretion of glutamate into the apoplast<sup>54</sup> strongly suggests that secreted glutamate might activate GLRs also in plant tissues. In the future, it will be important to study possible roles of GLRs and CNGCs, as well as of secreted L-glutamate and D-serine, in the context of secretory-active polar plant cells, organized into longitudinal cell files of plant organs such as root apex; generating its own bioelectric fields.

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